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Treatment of allergic rhinitis with intranasal infusion of botulinum toxin type A in mice

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Running title: Treatment of allergic rhinitis with botulinum toxin type A

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Abstract

Aims: To determine whether intranasal infusion of botulinum toxin type A (BTX-A) relieves symptoms of ovalbumin (OVA)-induced allergic rhinitis (AR) and reduces nasal inflammation in mice.

Main methods: AR was induced via intraperitoneal injection of OVA followed by daily intranasal challenge with OVA. Five weeks after the initiation of OVA sensitization, nasal cavities were exposed to a single intranasal infusion of BTX-A. The behavior of mice was observed before and 1, 3, 5, 7, 14, 21, 28, days after infusion. Mice were sacrificed after 28 days and late histological findings were examined. PBS was administered to control mice.

Results: On Day 3, the frequency of typical AR symptoms, including sneezing and nose scratching, significantly decreased in the BTX-A-treated group (n=6) compared to the control group (n=6). Although the AR-inhibiting effects of BTX-A persisted until Day 21, AR symptoms re-appeared in response to daily OVA stimulation. Histological findings of the nasal mucosa also improved following BTX-A administration. Although capillary dilatation and eosinophil infiltration decreased by Day 3, these effects disappeared by Day 28. In contrast, the number and size of the secretory glands in the nasal mucosa did not change following BTX-A administration. PBS had no effect on nasal symptoms or histology.

Conclusions: Topical treatment with BTX-A efficiently and temporarily ameliorates AR symptoms. Intranasal infusion does not cause pain or bleeding, and the effects of a single

infusion of BTX-A last for at least three weeks. This treatment might be a promising therapeutic strategy for the treatment of AR.

Key words: botulinum toxin, nasal drip infusion, experimental allergic rhinitis, ovalbumin, eosinophil infiltration

Introduction

Allergic rhinitis (AR) is an IgE-mediated disease of the nasal mucosa that usually occurs after exposure to allergens such as cedar pollen, house dust mites, and animal hair. In Japan, the incidence of AR was approximately 39.4% in the 2008 Practical Guidelines for the Management of Allergic Rhinitis in Japan.¹⁾ Current treatment of this disease involves the avoidance of allergens when possible, which is often difficult and impractical. To prevent or reduce the symptoms of AR, various medications have been developed such as corticosteroids, antihistamines, decongestants, anticholinergics, and leukotriene receptor antagonists. Few of these, however, enable the complete relief of symptoms without complications. Further, these medications usually require frequent administration once- or twice-daily for a relatively long period of time, thus deteriorating the quality of life (QOL) of patients.

Botulinum toxin type A (BTX-A) is a neurotoxin produced by the bacterium *Clostridium botulinum*. According to Burgen et al., BTX-A blocks signal transmission at the neuromuscular and neuroglandular junctions by decreasing acetylcholine release from presynaptic nerve endings.²⁾ BTX-A is now widely used to treat various neuromuscular disorders, such as strabismus, blepharospasm, achalasia, and facial spasm.³⁾ BTX-A has also been applied to cosmetics^{4),5)} and the prevention of gustatory sweating.⁶⁾ Due to this anticholinergic activity, BTX-A is considered a promising treatment for the attenuation of AR

symptoms. Various application procedures have been proposed, including injection into the nasal turbinate or septum,⁷⁾⁻¹¹⁾ placement of a BTX-A-containing cotton or gauze on the nasal mucosa,^{12),13)} and placement of gel formula on the nasal turbinate.¹⁴⁾ Nasal drip infusion is considered the most convenient way to administer BTX-A into the nasal cavity without causing pain or bleeding. To our knowledge, however, this procedure has not been reported for BTX-A.

Here, we investigated whether nasal drip infusion of BTX-A could attenuate the nasal symptoms and reduce inflammatory responses of the nasal mucosa in a murine model of AR. We also investigated how long the effects of a single intranasal infusion persisted by successive observation of the behavior of mice.

Materials and methods *Animals*

Six-week-old female Balb/c mice (Charles River Laboratories Japan, Inc., Yokohama, Japan), weighing 25 to 35 g were housed in cages at a temperature of 18 to 22 °C under a 12-h light/dark cycle. Mice were allowed free access to food and water until the end of the experiment.

The present study was conducted in accordance with our institute's guidelines for animal care under approval by the Ethics Committee of Ehime University Graduate School of Medicine. Every effort was made to minimize the number of animals involved and their suffering.

Induction of AR and administration of BTX-A

Figure 1 shows the protocol of this experiment. Mice received an intraperitoneal injection of 20 µg of ovalbumin (OVA) and 100 µl of aluminium hydroxide with 0.2 ml of saline as an adjuvant at three time points over 2 weeks (Days 0, 7, and 14). Mice were then administered 10 µl of OVA via the bilateral nasal cavities twice a day for 3 weeks to facilitate nasal sensitization. Following sensitization, mice exhibited typical symptoms of AR such as sneezing, watery rhinorrhea, and nose scratching when exposed to OVA. Sensitization was confirmed by measuring serum OVA-specific IgE using an enzyme-linked immunosorbent assay (ELISA).

Three weeks after the end of OVA sensitization, the OVA-specific serum IgE level of

the sensitized group was 82.8 ± 35.6 units/ml ($n=6$), while that of the untreated group was 11 ± 1.4 units/ml ($n=6$). At this point, OVA-sensitized mice were divided into a BTX-A group ($n=12$) and PBS group ($n=12$). In the BTX-A group, 10 μ l of BTX-A was applied once to each nasal cavity using a micropipette, and in the PBS group, 10 μ l of PBS was applied. Mice were then returned to their cages. Daily exposure to OVA was continued until the end of the study.

Day 1 was defined as the first day of BTX-A treatment, or 5 weeks post-OVA sensitization.

Evaluation of allergic responses

Responses to local OVA stimuli were observed before and 1, 3, 5, 7, 14, 21, and 28 days after BTX-A treatment. After nasal stimulation with OVA, episodes of sneezing and nose scratching were counted over a 10-min period from 20 to 30 min.

Histological study

After successive observation, animals were sacrificed on Day 3 to examine early histological findings of the nose and on Day 28 to examine late histological findings of the nose. Six mice from both the BTX-A and PBS groups were subjected to early and late histological study.

Under deep anesthesia with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg), mice were subjected to transcardial perfusion with saline, followed by 4% paraformaldehyde. Following decapitation, the skull—including the

brain—was fixed with 4% paraformaldehyde at 4 °C overnight before being decalcified with EDTA, dehydrated with a series of alcohol washes, and embedded in paraffin. The specimen was then serially sectioned into 5- μ m-thick slices and subjected to hematoxylin and eosin (HE) staining. Periodic Acid Schiff (PAS) staining was used for observation of nasal glandular tissues.

The thickness of the nasal mucosa was measured using DP controller software version 03.02 (Olympus, Tokyo, Japan). Measurements were taken at five different sites, and mean values compared between the BTX-A and PBS groups.

Statistical analysis

Results were analyzed using an unpaired *t*-test. All statistical analyses were conducted using IBM SPSS statistical software (SPSS, Chicago, IL). *P*-value less than 0.05 was considered significant.

Results

Figure 2 shows sequential changes in OVA-induced sneezing over a 10-min period following nasal drip infusion of BTX-A. The number of sneezing episodes significantly decreased following the application of BTX-A on Days 1, 3, 5, 7, 14, and 21 ($P<0.01$, or $P<0.001$). Prevention of allergic responses following BTX-A administration persisted for 3 weeks and then gradually subsided. Similar results were also observed for the frequency of nose scratching, as shown in Figure 3. These findings suggest that the allergic reactions evoked by OVA were significantly alleviated by a single intranasal infusion of BTX-A for at least 3 weeks.

Figure 4 shows representative images of the inferior turbinate mucosa in response to OVA sensitization before and at 3 and 28 days after BTX-A treatment. Before treatment, the nasal mucosa was swollen due to OVA sensitization. Capillary vessels were extremely dilated with infiltration of eosinophils into the submucosal layer. On Day 3, these histological changes were dramatically improved by administration of BTX-A. On Day 28, however, mucosal swelling and eosinophil infiltration reappeared in response to daily OVA stimulation. The thickness of the inferior turbinate mucosa on Days 3 and 28 are summarized in Figure 5. On Day 3, the mucosa was significantly thinner compared to controls ($P<0.05$). On Day 28, however, swelling of the mucosal membrane returned following sensitization with OVA. Figure 6 shows the number of eosinophils per square-millimeter in the inferior turbinate

mucosa. As expected, treatment with BTX-A reduced eosinophil infiltration on Day 3 ($P<0.05$) but proved ineffective by Day 28.

Figure 7 shows the nasal mucosa stained with PAS. On Day 3, the number and size of the secretory glands in the nasal mucosa did not differ between the BTX-A and control groups, although the volume of nasal secretions decreased.

Discussion

As shown in the present study, a single intranasal infusion of BTX-A alleviated the symptoms of OVA-induced allergic reactions of the nose for 3 weeks. In addition, treatment with BTX-A reduced eosinophil infiltration and thickness of the inferior turbinate mucosa on Day 3, but proved ineffective by Day 28. BTX-A is believed to control AR through blockage of signal transmission at the neuromuscular and neuroglandular junctions and suppression of neuropeptide release such as substance P (SP) and vasoactive intestinal peptide (VIP) from the trigeminal and parasympathetic nerve endings in response to stimulation by allergens.¹²⁾ SP and VIP released in the nasal mucosa stimulate secretory glands, which are present in a high density in the nasal cavity of mice. As BTX-A can suppress the release of SP and VIP, the staining of these substances in the nasal mucosa was weak after treatment.¹²⁾ According to Rhorback et al., BTX-A injection in the nasal turbinate caused degeneration of the nasal glands and ducts via induction of apoptosis.¹³⁾ In their study, mice exhibited almost normal glandular tissue with few apoptotic nuclei in the nasal cavity at three months after treatment. In our study, no nasal glandular degeneration was observed when symptoms of AR reappeared on Day 28, possibly due to the application of BTX-A via nasal drip infusion instead of injection into the nasal mucosa and the limited observation period.

Treatment of AR with BTX-A has been attempted through various procedures. For example, Unal et al. injected BTX-A into bilateral nasal mucosa in patients with AR and

reported that a single injection of BTX-A attenuated the nasal symptoms of AR for up to 8 weeks.⁸⁾ Yang et al. compared the therapeutic effects of BTX-A or steroids following injection into the inferior turbinates of patients.¹⁰⁾ They concluded that BTX-A was more effective than steroids in relieving AR symptoms, particularly for suppressing nasal obstruction and rhinorrhea. Abtahi et al. compared the effects of BTX-A injection into the nasal septum and those into the inferior turbinate in AR patients.¹¹⁾ Although both methods effectively reduced AR symptoms, injection into the nasal septum was safer and easier than injection into the inferior turbinate.

Alleviation of AR symptoms can also be achieved through the use of BTX-A-soaked sponges. Rohrbach et al. introduced BTX-A-soaked sponges to each nostril using bayonet forceps,³⁾ which remained in the nose for 1 h. After exposure, a marked reduction in nasal secretions was observed, but nasal congestion was unchanged. They concluded that such a local treatment was minimally invasive and favorable for treatment of AR for as much as 12 weeks, even in patients with conventional therapy-resistant symptoms.

Nasal drip infusion is a frequently utilized procedure for various medications such as steroids, antibiotics, and decongestants in the nasal mucosa. Given that BTX-A is easily absorbed through the nasal mucosa, patients can perform this procedure independently. In contrast to the effects of steroids, those of a single intranasal infusion of BTX-A persisted for at least 3 weeks. When BTX-A is applied excessively, however, paralysis of the

oropharyngeal muscles is a possible adverse effect. We are now planning a clinical trial of nasal drip infusion of BTX-A in patients with seasonal and perennial AR.

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Conclusions

Intranasal infusion of BTX-A efficiently attenuates the symptoms of OVA-induced AR in mice. This treatment also reduces eosinophil infiltration and capillary dilatation of the nasal mucosa. As the effects of a single intranasal infusion of BTX-A persist for at least 3 weeks, this modality might be a promising method for treating AR.

Figure legends

Figure 1. Experimental protocol. Nasal allergy was induced by three intraperitoneal injections of OVA and aluminum hydroxide over the course of two weeks, followed by daily nasal challenge with OVA. Five weeks post-OVA sensitization, a single intranasal infusion of BTX-A was administered to reduce allergic responses induced by OVA.

Figure 2. Sequential changes in OVA-induced nasal symptoms after nasal drip infusion of BTX-A. A, Number of sneezes per 10 min; B, Number of nose scratches per 10 min. Incidence of sneezing and nose scratching significantly decreased until 3 weeks post-BTX-A administration.

Figure 3. Representative images of the inferior turbinate mucosa stained with HE. A, Pre-BTX-A treatment; B, Day 3 post-BTX-A treatment; C, Day 28 post-BTX-A treatment. Post-BTX-A treatment, capillary dilatation, edema of the interstitial layer, and eosinophil infiltration were initially reduced but reappeared by Day 28.

Figure 4. Thickness of inferior turbinate mucosa on Days 3 and 28. Nasal mucosa was thinner on Day 3 than pre-treatment but returned to pre-treatment levels by Day 28.

Figure 5. Number of eosinophils per square-millimeter on Days 3 and 28. Number of eosinophils per square-millimeter significantly decreased on Day 3, but no marked differences were noted between BTX-A and PBS groups on Day 28.

Figure 6. PAS staining findings of the nasal mucosa on (A) Day 3 and (B) 28. Number and size of the nasal glands did not markedly differ between BTX-A and PBS groups.

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Figure 1.

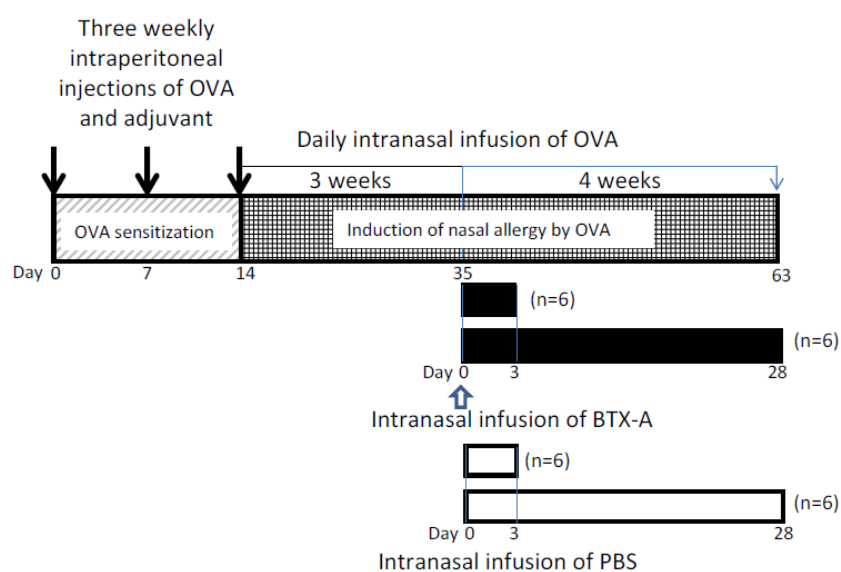


Figure 2A.

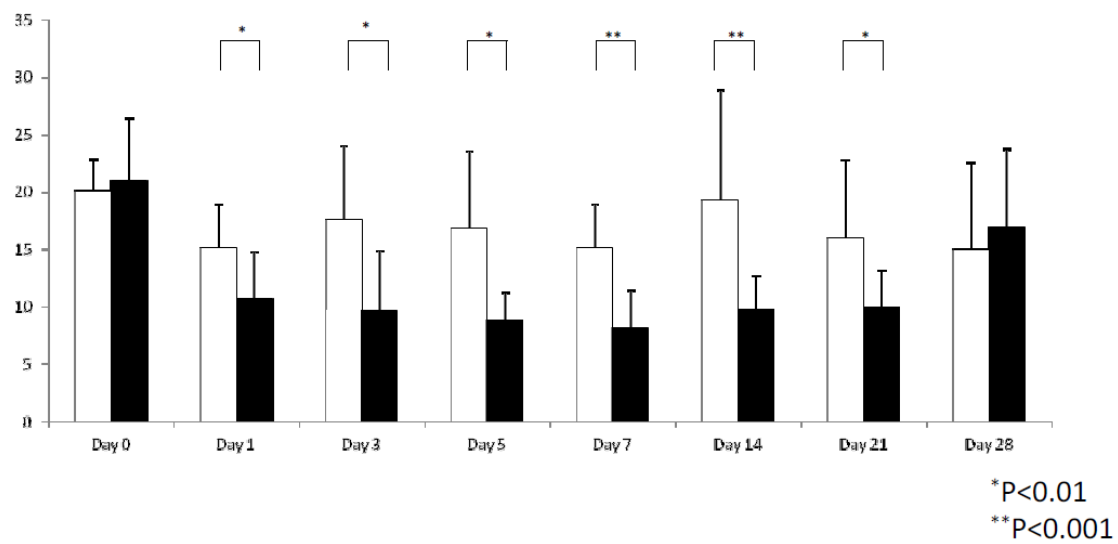
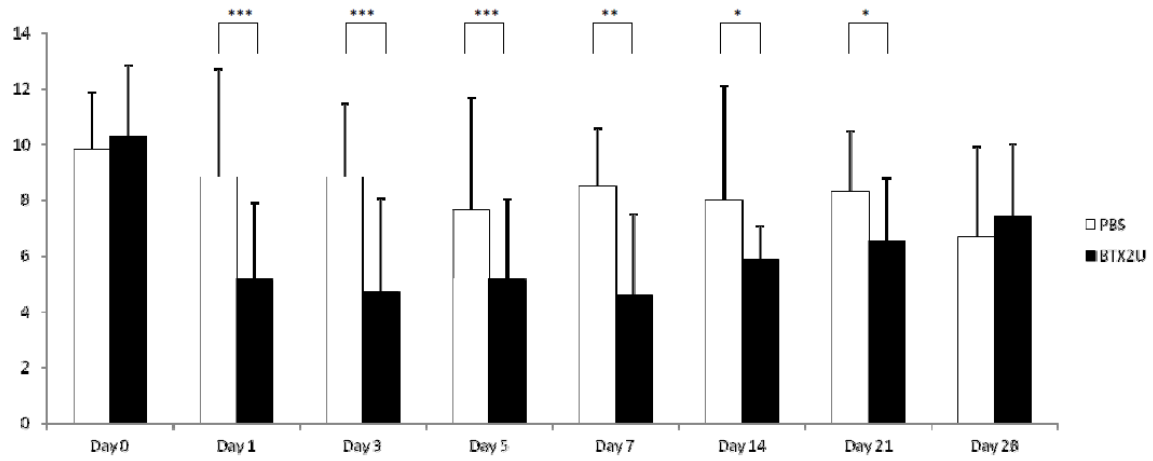


Figure 2B.



*P<0.05

**P<0.01

***P<0.001

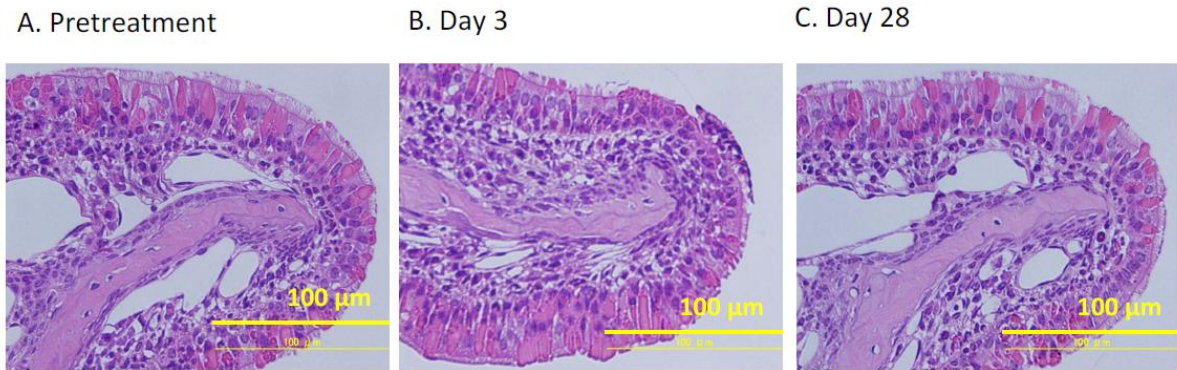
Figure 3.

Figure 4.

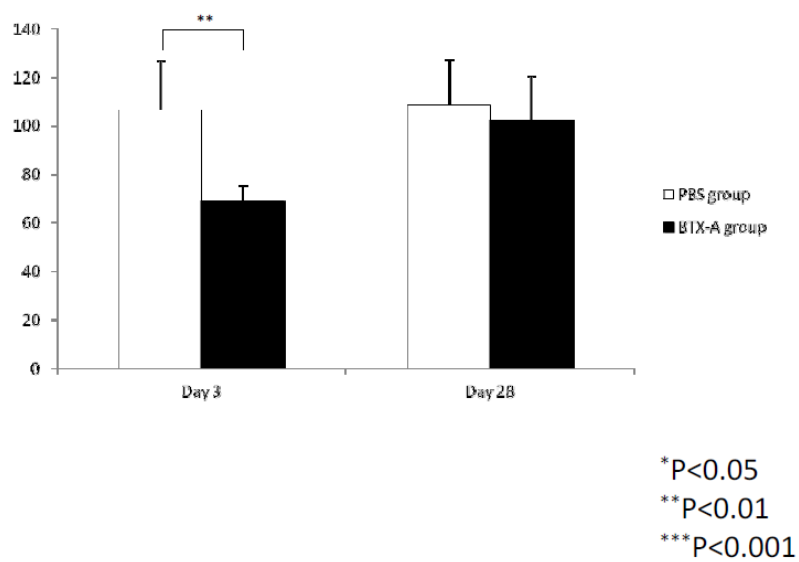


Figure 5.

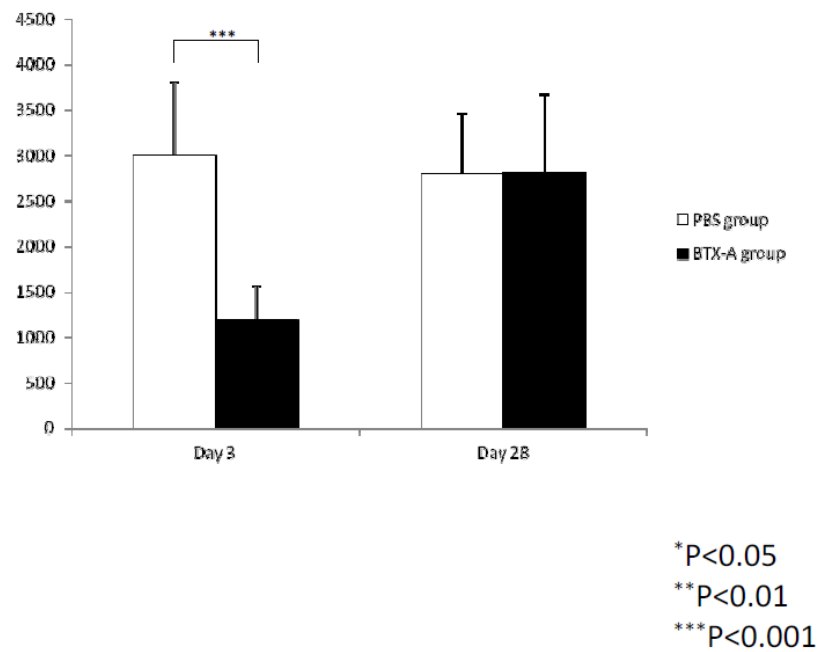
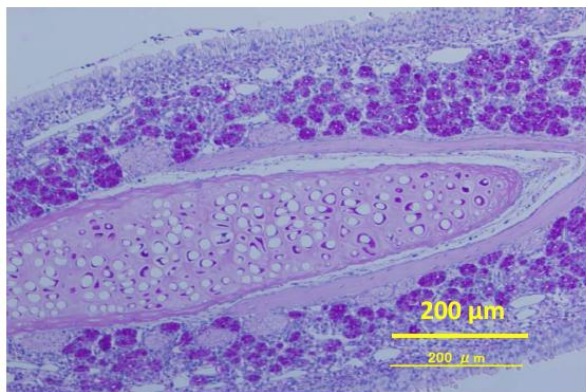
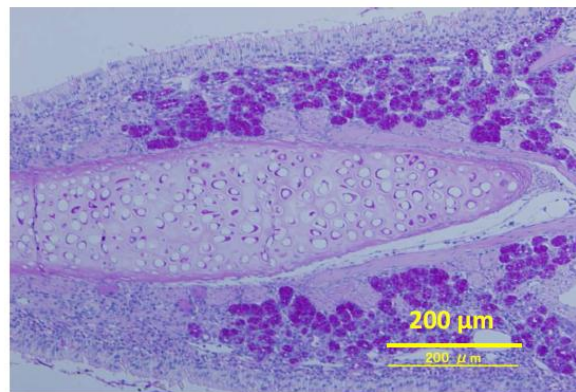


Figure 6.



B. Day 3



C. Day 28